4/7/1 (Item 1 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

13329803 BIOSIS Number: 99329803
A conformational epitope in the N-terminus of the Escherichia coli heat-stable enterotoxins is involved in receptor-ligand interactions
Garrett B M; Visweswariah S S
Centre Reproductive Mol. Endocrinol., Indian Inst. Sci., Bangalore 560012, India
Biochimica et Biophysica Acta 1317 (2). 1996. 149-154.
Full Journal Title: Biochimica et Biophysica Acta
ISSN: 0006-3002
Language: ENGLISH
Print Number: Biological Abstracts Vol. 103 Iss. 003 Ref. 032917
The heat-stable enterotoxins are a family of low molecular weight, cysteine rich peptide toxins which are one of the major causes of watery diarrhea in children and adults. These toxins bind to a cell surface receptor in intestinal cells and mediate their action through elevation of intracellular cyclic GMP. We have generated a monoclonal antibody to these peptide toxins which is able to neutralise the activity of the peptides in a human.colonic cell line, the T84 cell line. The monoclonal antibody, ST:G8, appears to be directed to an epitope distinct from antibodies previously generated, and prior incubation of this antibody, or Fab generated from this antibody, with full length STh and STp peptides prevents cGMP accumulation in T84 cells. This inhibition is a direct result of the antibody preventing binding of the peptides to the receptor. ST:G8 Mab does not recognize a 13-mer biologically active analog of STp, comprising the core sequence of STp peptide, suggesting that it is directed to a region in the N-terminus of the peptides, which may modulate receptor interaction/activation. The antibody recognizes a conformational epitope in the ST peptides, since reduction and carboxyamidation of ST abolishes antibody cross-reactivity. Differential cross-reactivity of the Mab with STh and STp peptides, which differ markedly only in their N-termini, suggests that this antibody recognizes a distinct conformation in the two peptides, which is essential for receptor interaction.

4/7/2 (Item 2 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

13231039 BIOSIS Number: 99231039
Expression of the extracellular domain of the human heat-stable enterotoxin receptor in Escherichia coli and generation of neutralizing antibodies
Nandi A; Mathew R; Visweswariah S S
Cent. Reprod. Biol. and Molecular Endocrinol., Indian Inst. Sci., Bangalore 560012, India
Protein Expression and Purification 8 (2). 1996. 151-159.
Full Journal Title: Protein Expression and Purification
ISSN: 1046-5928
Language: ENGLISH
Print Number: Biological Abstracts Vol. 102 Iss. 010 Ref. 146669
The entire extracellular domain of the human heat-stable enterotoxin (ST) receptor as well as a truncated N-terminal domain were cloned as glutathione S-transferase fusion proteins and expressed in Escherichia

coli. The recombinant fusion proteins were purified from both the cytosol and the inclusion body fractions by selective detergent extraction followed by glutathione-agarose affinity chromatography. The purified protein, corresponding to the entire extracellular domain, bound the stable toxin peptide with an affinity comparable to that of the native receptor characterized from the human colonic T84 cell line. No binding was observed with the N-terminal truncated fragment of the receptor under similar conditions. Polyclonal antibodies were raised to the entire extracellular domain fusion protein as well as the truncated extracellular domain fusion protein, and the antibodies were purified by affinity chromatography. Addition of the purified antibodies to T84 cells inhibited ST binding and abolished ST-mediated cGMP production, indicating that critical epitopes involved in ligand interaction are present in the N-terminal fragment of the receptor. Purified antibodies recognized a single protein of M-r 160,000 Da on Western blotting with T84 membranes, corresponding to a size of the native glycosylated receptor in T84 cells. These studies are the first report of the expression, purification, and characterization of any member of the guanylyl cyclase family of receptors in E. coli and show that binding of the toxin to the extracellular domain of the receptor is possible in the absence of any posttranslational modifications such as glycosylation. The recombinant fusion proteins as well as the antibodies that we have generated could serve as useful tools in the identification of critical residues of the extracellular domain involved in ligand interaction.

4/7/3 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07392739 92310270
Interaction of heat-stable enterotoxins with human colonic (T84) cells: modulation of the activation of guanylyl cyclase.
Visweswariah SS; Shanthi G; Balganesh TS
Astra Research Centre, Bangalore, India.
Microb Pathog (ENGLAND) Mar 1992, 12 (3) p209-18, ISSN 0882-4010
Journal Code: MIC
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Heat-stable enterotoxins (ST) activate guanylyl cyclase in T84 cells, rapidly and specifically. Activation is monitored by cGMP production and occurs at lower concentrations of ST than required for eliciting fluid accumulation in suckling mice. Atrial natriuretic factor (ANF) neither activates guanylyl cyclase nor modulates the response to ST in T84 cells, indicating the absence of receptors for ANF on T84 cells. Monitoring the production of cGMP under conditions known to alter fluid accumulation in suckling mice is an accurate and quantifiable assay of ST activity and its interaction with the receptor. STs produced by Escherichia coli, Vibrio cholerae non-01 and Yersinia enterocolitica individually produce elevated levels of cGMP in T84 cells, but to differing extents, suggesting that this model system can be used to elucidate the different events of ST-receptor interactions at the molecular level.

4/7/4 (Item 1 from file: 156)
DIALOG(R)File 156:Toxline(R)

02257454 Subfile: CRISP-96-DK41324-06
REGULATION OF CHLORIDE CONDUCTANCE IN NORMAL & CF CELLS
GARDNER PI
STANFORD UNIVERSITY MED CTR, CVRB, CV-291, STANFORD, CA 94305-5332
Source: Crisp Data Base National Institutes Of Health
Language: ENGLISH
Document Type: Research
Spon. Agency: U.S. DEPT. OF HEALTH AND HUMAN SERVICES; PUBLIC HEALTH SERVICE; NATIONAL INST. OF HEALTH, NAT INST OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES
Contract Number: 5R01DK41324-06
Award Type: Grant
RPROJ/CRISP The primary control point for transepithelial electrolyte transport is C1- secretion across the apical membrane. Several types of external receptors, coupled to a variety of effector systems (CAMP, Ca2+, CGMP, PKC) mediate C1- secretion. Cystic fibrosis (CF) is characterized by abnormal C1- secretion in epithelial cells, with some regulatory pathways affected and others preserved. CAMP-dependent C1- secretion, induced by CAMP kinase-mediated phosphorylation of cystic fibrosis transmembrane conductance regulator (CFTR), is affected in CF. Ca2+-dependent C1- secretion, induced by CaM kinase-mediated phosphorylation of an independent C1- channel, is preserved in CF. Delineation of C1-transport pathways which are preserved in CF presents potential targets for therapeutic intervention. The overall goal of this proposal is, therefore, to investigate other regulatory pathways of C1- secretion that may ultimately serve to bypass the CF defect. The techniques employed will be patch clamp recording, supplemented by spectrofluorimetry and I125 efflux assays, of normal and CF-derived airway and intestinal epithelial cell lines. The specific aims include the investigation of' i) CGMP-dependent C1- secretion: to characterize currents mediated by heat stable enterotoxin (ST) in intestinal cells and by CPT-CGMP in airway cells; by use of coexpression of the ST receptor and CFTR in nonepithelial cells, to determine whether the pathway is CF-affected. ii) PKC-dependent C1- secretion: to determine by immunoblot the specific PKC isoforms expressed in epithelial cells; to characterize PKC-dependent regulation of C1- transport; to determine status in CF. iii) purinoreceptor pathway of C1- secretion; to determine the predominant receptor subtype by which adenosine and ATP analogues stimulate secretion; to investigate the responsible effector system; to determine status in CF.

4/7/5 (Item 1 from file: 340)
DIALOG(R)File 340:CLAIMS(R)/US PATENTS ABS

2812178 9703730
C/ METHODS OF DIAGNOSING COLORECTAL TUMORS AND METASTASIS THEREOF
Document Type: UTILITY
Inventors: Waldman Scott A (US)
Assignee: Jefferson, Thomas University Assignee Code: 06943

	Patent Number	Issue Date	Applic Number	Applic Date
Patent:	US 5601990	970211	US 305056	940913
Priority Applic:			US 305056	940913

Abstract:
In vitro methods of determining whether or not an individual has metastasized colorectal cancer cells are disclosed. In vitro methods of

determining whether or not tumor cells are colorectal in origin are disclosed. In vitro kits for practicing the methods of the invention and to reagents and compositions useful to practice the methods, for example as components in such in vitro kits of the invention are provided. Methods of and kits and compositions for analyzing tissue samples from the colon tissue to evaluate the extent of metastasis of colorectal tumor cells are disclosed.

Exemplary Claim:

1. An in vitro method of determining whether or not an individual has metastasized colorectal cancer cells comprising the steps of examining a sample of extraintestinal tissue and/or body fluids from an individual to determine whether ST receptor protein is being expressed by cells in said sample, wherein expression of ST receptor protein is indicative of the presence of metastasized colorectal cancer cells in said sample.

4/7/6 (Item 1 from file: 351)
DIALOG(R)File 351:DERWENT WPI

010277391 WPI Acc No: 95-178646/23
Related WPI Accession(s): 95-194370
XRAM Acc No: C95-082689
XRPX Acc No: N95-140284

Conjugated cpds. which specifically bind to colorectal cancer cells - comprise heat-stable toxin receptor binding moiety and active moiety which may be a therapeutic agent or a radioactive agent

Patent Assignee: (UYJE-) UNIV JEFFERSON THOMAS
Author (Inventor): WALDMAN S A
Number of Patents: 006
Number of Countries: 060
Patent Family:

CC Number	Kind	Date	Week	
WO 9511694	A1	950504	9523	(Basic)
AU 9481249	A	950522	9534	
US 5518888	A	960521	9626	
NO 9601706	A	960620	9634	
EP 734264	A1	961002	9644	
US 5601990	A	970211	9712	

Priority Data (CC No Date): US 305056 (940913); US 141892 (931026)
Applications (CC,No,Date): WO 94US12232 (941026); AU 9481249 (941026); WO 94US12232 (941026); NO 961706 (960426); WO 94US12232 (941026); EP 95900421 (941026)
Language: English
Designated States
(National): AM; AT; AU; BB; BG; BR; BY; CA; CH; CN; CZ; DE; DK; EE; ES; FI ; GB; GE; HU; JP; KE; KG; KP; KR; KZ; LK; LR; LT; LU; LV; MD; MG; MN; MW; NL; NO; NZ; PL; PT; RO; RU; SD; SE; SI; SK; TJ; TT; UA; US; UZ; VN
(Regional): AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; KE; LU; MC; MW; NL ; OA; PT; SD; SE; SZ; LI
Filing Details: AU9481249 Based on WO 9511694; EP0734264 Based on WO 9511694

Abstract (Basic): WO 9511694 A

Anew conjugated cpd. (I) comprises (1) an ST receptor binding moiety and (2) an active moiety which is a radiostable agent. ('ST' refers to heat-stable toxin produced by E.coli and other organisms.) Also claimed are pharmaceutical compsns. and methods of treatment or

radioimaging of metastasized colorectal cancer using (I); and a method of delivery of a nucleic acid molecule to intestinal tract cells using (I) in which the active moiety is the nucleic acid. Also claimed is an in-vitro method of determining whether or not an individual has metastasized colorectal cancer cells, or of determining whether a tumour cell is a colorectal tumour cell, both methods involving determining whether ST receptor protein is expressed by cells in a sample. Further claimed is a kit for carrying out the determination.

USE - (I) are used in methods for the detection, imaging and treatment of colorectal tumours, particularly metastasized tumours.

ADVANTAGE - The claimed cpds. may be used for the early diagnosis and treatment of large bowel cancer, improving the prognosis of an individual with the disease. Recent approaches involving the specific targeting of agents to tumour cells using monoclonal antibodies have a number of associated problems. The present invention allows specific binding to colorectal cancer cells which may improve methods of imaging and treating metastasized forms of said cells.

Dwg.0/0

Abstract (US): 9712 US 5601990 A

An in vitro method of determining whether or not an individual has metastasized colorectal cancer cells comprises examining a sample of extra-intestinal tissue and/or body fluids from an individual to determine whether ST receptor protein is being expressed by the cells in the sample, where the expression of ST receptor protein is indicative of the presence of metastasized colorectal cancer cells in the sample.

Dwg.0/0 9626 US 5518888 A

A method of imaging metastasised colorectal cancer cells in an individual comprising the steps of:

a) administering into the circulatory system of said individual, a diagnostically effective amount of a pharmaceutical composition comprising:

i) a pharmaceutically acceptable carrier or diluent, and,

ii) a conjugated compound comprising:

1) a ST receptor binding moiety; and,

2) an active moiety;

wherein said ST receptor binding moiety is a heat stable (ST) toxin peptide having less than 25 amino acids or fragments or derivatives thereof, wherein said heat stable toxin, fragments or derivatives thereof specifically bind to the ST receptor, and said active moiety is an imaging agent which can be detected in said individual's body; and

b) detecting localization and accumulation of said imaging agent in said individual's body.

Dwg.0/0

Derwent Class: B04; D16; K08; S03;

Int Pat Class: A61K-038/10; A61K-051/00; A61K-051/08; G01N-033/53; G01N-033/532; G01N-033/534; G01N-033/566; G01N-033/574

Derwent Registry Numbers: 0165-U; 0180-U; 1166-U; 1215-U; 1257-U; 2028-U

4/7/7 (Item 1 from file: 434)

DIALOG(R)File 434:Scisearch(R) Cited Ref Sci

14708280 Genuine Article#: UF518 Number of References: 23

Title: RESPONSES OF PORCINE CORPORA-LUTEA TO SOMATOTROPIN ADMINISTRATION DURING PREGNANCY

Author(s): YUAN W; STERLE JA; CANTLEY TC; LAMBERSON WR; DAY BN; LUCY MC
Corporate Source: UNIV MISSOURI,DEPT ANIM SCI,ANIM SCI RES CTR 164/COLUMBIA//MO/65211; UNIV MISSOURI,DEPT ANIM SCI,ANIM SCI RES CTR 164/COLUMBIA//MO/65211
Journal: JOURNAL OF ANIMAL SCIENCE, 1996, V74, N4 (APR), P873-878
ISSN: 0021-8812
Language: ENGLISH Document Type: ARTICLE
Abstract: The effects of somatotropin (ST) on functions of porcine corpora lutea (CL) during pregnancy were investigated. Twenty-four crossbred (Yorkshire/Landrace) gilts from d 30 to 43 of pregnancy were injected daily with 5 mg of recombinant porcine somatotropin (rpST; n = 12) or 1 mL of saline (control, n = 12). Blood was collected on d 30, 37, and 43 for analyses of plasma progesterone. Gilts were killed on d 44 of pregnancy, and mRNA were isolated from CL, ovary, and liver. Messenger RNA expression for LH receptor, FSH receptor, ST receptor, 3 beta-hydroxysteroid dehydrogenase (3 beta-HSD), and cytochrome P-450 side-chain cleavage enzyme (SCC) were measured. Liver, CL, and ovary contained a 4.7-kb mRNA of ST receptor, but the liver contained more mRNA for ST receptor than did CL or ovary (.97 +/- .18, .47 +/- .04, and .25 +/- .04 units, respectively). There were two variants of LH receptor mRNA in CL (6.8 and 4.4 kb). The CL also contained a 1.8-kb mRNA of SCC and a 1.7-kb mRNA of 3 beta-HSD. No FSH receptor mRNA was detected in CL of the pig. The rpST treatment did not affect the mRNA level of ST receptor, 3 beta-RSD, SCC, or 4.4-kb mRNA of the LH receptor. The 6.8-kb mRNA for the LH receptor was decreased ($P < .05$) by rpST (.56 +/- .04 vs .78 +/- .05 units). Furthermore, concentrations of plasma progesterone decreased ($P < .001$) in gilts treated with rpST. Decreased luteal function was associated with decreased expression of LH receptor in rpST-treated gilts. The luteotropic effects of ST observed in vitro dq not necessarily occur in vivo when gilts are administered rpST during pregnancy.

4/7/8 (Item 2 from file: 434)
DIALOG(R)File 434:Scisearch(R) Cited Ref Sci

13126983 Genuine Article#: NR296 Number of References: 46
Title: INDUCTION OF HEAT-STABLE ENTEROTOXIN RECEPTOR ACTIVITY BY A HUMAN ALU REPEAT
Author(s): ALMENOFF JS; JURKA J; SCHOOLNIK GK
Corporate Source: DUKE UNIV,MED CTR,DIV INFECT DIS,RM 1558,DUKE S,BLUE ZONE/DURHAM//NC/27710; STANFORD UNIV,MED CTR,HOWARD HUGHES MED INST/STANFORD//CA/94305; LINUS PAULING INST SCI & MED/PALO ALTO//CA/94304
Journal: JOURNAL OF BIOLOGICAL CHEMISTRY, 1994, V269, N24 (JUN 17), P 16610-16617
ISSN: 0021-9258
Language: ENGLISH Document Type: ARTICLE
Abstract: The heat-stable enterotoxins (ST) elaborated by enterotoxigenic Escherichia coli are a family of small cysteine-rich peptides that bind to specific epithelial receptors in the mammalian intestine, causing a secretory diarrhea. The expression of ST receptors is tightly regulated; they are found primarily in intestine, and their expression is developmentally modulated. One receptor for ST has been cloned, and its cDNA encodes a similar to 120-kDa particulate guanylyl cyclase (guanylyl cyclase-C). Recent studies suggest that there are additional ST receptors that are not homologous to guanylyl cyclase-C. We used an

expression cloning strategy to identify intestinal mRNAs that lead to expression of ST receptor activity in transfected cells. Using an ST-specific affinity panning system, we identified a novel 1891-base pair cDNA that does not encode a receptor protein, but instead, consists primarily of untranslated sequence. This cDNA induced receptor activity in both COS and 293 embryonic kidney cells. Northern analysis of the T84 human intestinal cell line, from which this cDNA was cloned, suggests that it is part of a 7.8 kilobase mRNA transcript. This transcript was also identified in human small intestine and colon, as well. as in several extraintestinal tissues. Functional analysis of subcloned fragments reveals that ST binding activity is induced by a 457-base pair human Alu repetitive sequence within the cDNA and that the phenoytpe is independent of orientation. These findings suggest that a human Alu element induces expression of a unique ST receptor by a transacting mechanism. An unrelated Alu-rich genomic clone did not confer ST binding, suggesting that there may be structural and functional specificity within individual Alu sequences.

4/7/9 (Item 3 from file: 434)
DIALOG(R)File 434:Scisearch(R) Cited Ref Sci

12366476 Genuine Article#: LF036 Number of References: 40
Title: LIGAND-BASED HISTOCHEMICAL-LOCALIZATION AND CAPTURE OF CELLS EXPRESSING HEAT-STABLE ENTEROTOXIN RECEPTORS
Author(s): ALMENOFF JS; WILLIAMS SI; SCHEVING LA; JUDD AK; SCHOOLNIK GK
Corporate Source: STANFORD UNIV,BECKMAN CTR MOLEC & GENET MED,HOWARD HUGHES MED INST/STANFORD//CA/94305; SRI INT,DIV LIFE SCI/MENLO PK//CA/94025; VANDERBILT UNIV,MED CTR,SCH MED,DEPT PATHOL/NASHVILLE//TN/37232
Journal: MOLECULAR MICROBIOLOGY, 1993, V8, N5 (MAY), P865-873
ISSN: 0950-382X
Language: ENGLISH Document Type: ARTICLE
Abstract: The heat stable enterotoxins (ST) of enterotoxigenic Escherichia coli (ETEC) cause diarrhoea by binding specific intestinal receptors. Precise histochemical localization of ST receptors could provide more information about the pathophysiology of secretory diarrhoea and the role of ST receptors in normal biology. To accomplish this, we quantitatively coupled biotin to the N-terminus of ST1b using biotin-X-X-N-hydroxysuccinimide ester. The derivatized toxin (BST) has an apparent K(d) of 11.7+/-10 nM for rat brush border receptors. We used BST in an affinity panning cell-capture system, to validate its ability to discriminate between receptor-positive and receptor-negative cells. Cell lines expressing ST receptors (human colon carcinoma T84, and COS cells transfected with guanylyl cyclase-C (GC-C) ST receptor cDNA) were captured to streptavidin and anti-biotin-coated plates with high efficiency and specificity. This system provides a novel approach to screening cells for the presence of unique ST-binding proteins. BST was then used with streptavidin-gold to demonstrate the cellular topography of ST receptors at the light microscopic level. Villus enterocytes were intensely stained, but only a faint signal was observed in upper crypts of rat small intestine. Thus, a gradient of increasing receptor density was seen as upper crypt cells matured into villus enterocytes. Higher magnification revealed that ST receptors are.concentrated at the apical aspect of villus enterocytes. Recently, guanylin, a putative endogenous ligand for ST receptors, has been localized to Paneth cells, at the base of intestinal crypts. Thus, ST receptors are concentrated in villus enterocytes, while guanylin

appears to be produced at the base of the crypts. This topographical arrangement suggests that there are autocrine or paracrine pathways by which ST receptors interact with endogenous ligands.

8/7/1 (Item 1 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

12185968 BIOSIS Number: 98785968
The significance of Ser-1029 of the heat-stable enterotoxin receptor (STaR): Relation of STa-mediated guanylyl cyclase activation and signaling by phorbol myristate acetate
Wada A; Hasegawa M; Matsumoto K; Niidome T; Kawano Y; Hidaka Y; Padilla P I; Kurazono H; Shimonishi Y; Hirayama T
Inst. Tropical Med., Nagasaki Univ., 1-12-4 Sakamoto, Nagasaki 852, Japan
FEBS Letters 384 (1). 1996. 75-77.
Full Journal Title: FEBS Letters
ISSN: 0014-5793
Language: ENGLISH
Print Number: Biological Abstracts Vol. 101 Iss. 011 Ref. 153160
To characterize Ser-1029 in STaR at a consensus sequence of phosphorylation site by PKC, two mutants of mS1029A with replacement of Ser-1029 to Ala-1029 and C-DELTA-1029 lacking 22 amino acids including Ser-1029 were prepared. Preincubation of the wild type-STaR (wt-STaR) transfectant with 1 mu-M PMA caused additional STa-mediated guanylyl cyclase (GC) activation compared to control, whereas the mS1029A- and C-DELTA-1029-transfected cells did not show a similar enhanced GC activation by PMA. After metabolic labeling with (32P)phosphate, transfected cells with wt-STaR and mutants were incubated with 1 mu-M PMA. Subsequent 32P-radiolabeled proteins were immunoprecipitated using anti-STaR antibody, and analyzed by autoradiography after separation on SDS-PAGE. The immunoprecipitated wt-STaR but not mS1029A and C-DELTA-1029 had a significant radioactivity. These results suggest that the effect of PMA on wt-STaR transfectants may be caused by phosphorylation of Ser-1029. The C-DELTA-1012 mutant, with further truncation (Gln-1012-Phe-1050) of the carboxy terminus, did not show STa-mediated GC activation. Based on these data, these 17 amino acids (Gln-1012-Ala-1028), essential for signaling of GC activation by STa, have an abundance of basic amino acids which might be functionally influenced by phosphorylation of Ser-1029.

8/7/2 (Item 2 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

12079922 BIOSIS Number: 98679922
Potent immunogenicity of the B subunits of Escherichia coli heat-labile enterotoxin: Receptor binding is essential and induces differential modulation of lymphocyte subsets
Nashar T O; Webb H M; Eaglestone S; Williams N A; Hirst T R
Research School Biosciences, University Kent, Canterbury, Kent CTG2 7NJ, UK
Proceedings of the National Academy of Sciences of the United States of America 93 (1). 1996. 226-230.
Full Journal Title: Proceedings of the National Academy of Sciences of the United States of America
ISSN: 0027-8424
Language: ENGLISH
Print Number: Biological Abstracts Vol. 101 Iss. 006 Ref. 080203
The importance of receptor binding in the potent immunogenicity of Escherichia coli heat-labile enterotoxin B subunit (EtxB) was tested by

comparing its immunogical properties with those of a receptor binding mutant, EtxB(G33D). Subcutaneous immunization of EtxB(G33D) resulted in 160-fold reduction in antibody titer compared with wild-type EtxB, whereas its oral delivery failed to provoke any detectable secretory or serum anti-B subunit responses. Moreover, the two proteins induced strikingly different effects on lymphocyte cultures in vitro. EtxB, in comparison with EtxB(G33D), caused an increase in the proportion of B cells, many of which were activated (CD25+); the complete depletion of CD8+ T cells; an increase in the activation of CD4+ T cells; and an increase in interleukin 2 and a decrease in interferon gamma. These data indicate that EtxB exerts profound effects on immune cells, suggesting that its potent immunogenicity is dependent not only on efficient receptor-mediated uptake, but also on direct receptor-mediated immunomodulation of lymphocyte subsets.

8/7/3 (Item 3 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

9146817 BIOSIS Number: 93131817
CHARACTERIZATION OF THE RECOMBINANT HUMAN RECEPTOR FOR ESCHERICHIA-COLI HEAT-STABLE ENTEROTOXIN
DE SAUVAGE F J; HORUK R; BENNETT G; QUAN C; BURNIER J P; GOEDDEL D V
GENENTECH INC., 460 POINT SAN BRUNO BLVD., SOUTH SAN FRANCISCO, CALIF. 94080.
J BIOL CHEM 267 (10). 1992. 6479-6482. CODEN: JBCHA
Full Journal Title: Journal of Biological Chemistry
Language: ENGLISH
We report here the molecular characterization of a recombinant cell line (293-STaR) expressing the heat-stable enterotoxin receptor (STaR) from human intestine. We have compared the 293-STaR cell line with the human colonic cell line T84 that endogenously expresses STa binding sites. Scatchard analysis of displacement binding studies revealed a single STa binding site with an affinity (Ki) of 97 pM in 293-STaR compared with 55 pM in T84 cells. Saturation isotherms of STa binding gave a Kd of 94 pM for the cloned receptor expressed in 293 cells and 166 pM for the receptor present in T84 cells. Kinetic measurements of STa binding to 293-STaR gave an association rate constant, K1, of 2.4 .times. 108 M-1 min-1 and a dissociation rate constant, K2, of 0.016 min-1. The half-time of dissociation was 43 min, and the Kd calculated from the ratio of the kinetic constants was 67 pM. The pH profile of STa binding showed that the number of STa binding sites is increased 3-fold at pH 4.0 compared with pH 7.0, with no effect on binding affinity. A polyclonal antibody directed against the extracellular domain of STaR immunoprecipitated two proteins of approximately 140 and 160 kDa from both 293-STaR and T84 cells. Cross-linking of 125I-STa to 293-STaR cells resulted in the labeling of proteins with a molecular mass of approximately 153, 133, 81, 68, 56, and 49 kDa, the two smallest being the more abundant. Similar results have been reported for the STaR present on rat brush border membranes. These data suggest that the STaR-guanylyl cyclase identified by molecular cloning is the only receptor for STa present in T84 cells.

8/7/4 (Item 4 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

8564481 BIOSIS Number: 92029481

HUMAN MAJOR HISTOCOMPATIBILITY COMPLEX CLASS-II-NEGATIVE COLON CARCINOMA CELLS PRESENT STAPHYLOCOCCAL SUPERANTIGENS TO CYTOTOXIC T LYMPHOCYTES EVIDENCE FOR A NOVEL ENTEROTOXIN RECEPTOR

DOHLSTEN M; HEDLUND G; SEGREN S; LANDO P A; HERMMANN T; KELLY A P; KALLAND T

KABI PHARMACIA THERAPEUTICS AB, IDEON, S-205 12 MALMO, SWEDEN.

EUR J IMMUNOL 21 (5). 1991. 1229-1234. CODEN: EJIMA

Full Journal Title: European Journal of Immunology

Language: ENGLISH

The staphylococcal enterotoxins (SE) bind to major histocompatibility complex (MHC) class II molecules on target cells and activate T cells expressing particular T cell receptor V.beta. sequences. In this report we demonstrate that SE bind to the MHC class II- SW620, Colo320DM and WiDr human colon carcinoma cell lines and direct cytotoxic T lymphocytes (CTL) to mediate strong target cell killing. Flow cytometry analysis, immunoprecipitation and Northern blotting experiments failed to demonstrate any surface expression of HLA-DR, HLA-DP and HLA-DQ isotypes on the SW620 colon carcinoma cell line, whereas abundant expression of these isotypes was seen on Raji cells. SEB and SEC1 were efficiently presented at picomolar concentration by the MHC class II- colon carcinoma cells and MHC class II+ Raji cells, whereas SEA and SED were preferentially presented on the MHC class II+ Raji cells. An anti-HLA-DR monoclonal antibody inhibited SEB-induced CTL targeting to Raji, but did not influence the killing of SW620 cells. Our data suggests the existence of functionally active SE-binding structures on human colon carcinoma cells which are distinct from the conventional MHC class II molecules. The possibility that these putative new SE receptors play a role in the enterotoxin action of SE must be considered.

8/7/5 (Item 5 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

5665670 BIOSIS Number: 33060691

ANTI-IDIOTYPIC ANTIBODIES AS STAPHYLOCOCCAL ENTEROTOXIN RECEPTOR PROBES ON MONKEY MAST CELLS

BAMBERGER U; SCHEUBER P H; SAILER-KRAMER B; HAMMER D K

MAX-PLANCK-INST. IMMUNIBIOLOGIE, POSTFACH 1169, D-7800 FREIBURG, FRG.

MEETING ON NEW ASPECTS OF ALLERGIC DISEASES, IMMUNOREGULATION AND IMMUNODEFIENCIES HELD AT THE 16TH SYMPOSIUM OF THE COLLEGIUM INTERNATIONALE ALLERGOLOGICUM, GOTEBORG, SWEDEN, AUGUST 17-21, 1986. INT ARCH ALLERGY APPL IMMUNOL 82 (3-4). 1987. 272-274. CODEN: IAAAA

Language: ENGLISH

8/7/6 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

9934339 EMBASE No: 96117598

The significance of Ser1029 of the heat-stable enterotoxin receptor (STaR): Relation of STa-mediated guanyl cyclase activation and signaling by phorbol myristate acetate

Wada A.; Hasegawa M.; Matsumoto K.; Niidome T.; Kawano Y.; Hidaka Y.; Padilla P.I.; Kurazono H.; Shimonishi Y.; Hirayama T.

Institute of Tropical Medicine, Nagasai University, 1-12-4 Sakamoto, Nagasaki 852 Japan

FEBS Letters (Netherlands) , 1996, 384/1 (75-77) CODEN: FEBLA ISSN: 0014-5793
LANGUAGES: English SUMMARY LANGUAGES: English
To characterize Ser1029 in STaR at a consensus sequence of phosphorylation site by PKC, two mutants of mS1029A with replacement of Ser1029 to Ala1029 and CDelta1029 lacking 22 amino acids including Ser1029 mere prepared. Preincubation of the wild type-STaR (wt-STaR) transfectant with 1 microM PMA caused additional STa-mediated guanylyl cyclase (GC) activation compared to control, whereas the mS1029A- and CDelta1029-transfected cells did not show a similar enhanced GC activation by PMA. After metabolic labeling with (32P)phosphate, transfected cells with wt-STaR and mutants were incubated with 1 microM PMA. Subsequent 32P-radiolabeled proteins were immunoprecipitated using anti-STaR antibody, and analyzed by autoradiography after separation on SDS-PAGE. The immunoprecipitated wt-STaR but not mS1029A and CDelta1029 had a significant radioactivity. These results suggest that the effect of PMA on wt-STaR transfectants may be caused by phosphorylation of Ser1029. The CDelta1012 mutant, with further truncation (Gln1012-Phe1050) of the carboxy terminus, did not show STa-mediated GC activation. Based on these data, these 17 amino acids (Gln1012-Ala1028), essential for signaling of GC activation by STa, have an abundance of basic amino acids which might be functionally influenced by phosphorylation of Ser1029.

8/7/7 (Item 1 from file: 76)
DIALOG(R)File 76:Life Sciences Collection

02036581 3910621
The significance of Ser super(1029) of the heat-stable enterotoxin receptor (STaR): Relation of STa-mediated guanylyl cyclase activation and signaling by phorbol myristate acetate
Wada, A.; Hasegawa, M.; Matsumoto, K.; Niidome, T.; Kawano, Y.; Hidaka, Y.; Padilla, P.I.; Kurazono, H.; Shimonishi, Y.; Hirayama, T.
Inst. Tropical Med., Nagasaki Univ., 1-12-4 Sakamoto, Nagasaki 852, Japan
FEBS LETT. vol. 384, no. 1, pp. 75-77 (1996)
ISSN: 0014-5793
DOCUMENT TYPE: Journal article LANGUAGE: ENGLISH
SUBFILE: Microbiology Abstracts B: Bacteriology; Toxicology Abstracts

To characterize Ser super(1029) in STaR at a consensus sequence of phosphorylation site by PKC, two mutants of mS1029A with replacement of Ser super(1029) to Ala super(1029) and C Delta 1029 lacking 22 amino acids including Ser super(1029) were prepared. Preincubation of the wild type-STaR (wt-STaR) transfectant with 1 mu M PMA caused additional STa-mediated guanylyl cyclase (GC) activation compared to control, whereas the mS1029A- and C Delta 1029-transfected cells did not show a similar enhanced GC activation by PMA. After metabolic labeling with [super(32)P]phosphate, transfected cells with wt-STaR and mutants were incubated with 1 mu M PMA. Subsequent super(32)P-radiolabeled proteins were immunoprecipitated using anti-STaR antibody, and analyzed by autoradiography after separation on SDS-PAGE. The immunoprecipitated wt-STaR but not mS1029A and C Delta 1029 had a significant radioactivity. These results suggest that the effect of PMA on wt-STaR transfectants may be caused by phosphorylation of Ser super(1029). The C Delta 1012 mutant, with further truncation (Gln super(1012)-Phe super(1050)) of the carboxy terminus, did not show STa-mediated GC activation. Based on these data, these 17 amino acids (Gln super(1012)-Ala super(1050)), essential for

signaling of GC activation by STa, have an abundance of basic amino acids which might be functionally influenced by phosphorylation of Ser super(1029).

8/7/8 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08990584 97133864
Phosphorylation and activation of the intestinal guanylyl cyclase receptor for Escherichia coli heat-stable toxin by protein kinase C.
Crane JK; Shanks KL
Division of Infectious Diseases, State University of New York at Buffalo 14214, USA.
Mol Cell Biochem (NETHERLANDS) Dec 20 1996, 165 (2) p111-20, ISSN 0300-8177 Journal Code: NGU
Contract/Grant No.: R29 DK 49410, DK, NIDDK
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The heat-stable enterotoxin STa of E. coli causes diarrhea by binding to and stimulating intestinal membrane-bound guanylyl cyclase, triggering production of cyclic GMP. Agents which stimulate protein kinase C (PKC), including phorbol esters, synergistically enhance STa effects on cGMP and secretion. We investigated whether PKC causes phosphorylation of the STa receptor in vivo and in vitro. Immunoprecipitation of the STa receptor-guanylyl cyclase was carried out from extracts of T84 colon cells metabolically labelled with [32P]-phosphate using polyclonal anti-STa receptor antibody. The STa receptor was phosphorylated in its basal state, and 32P content in the 150 kDa holoreceptor band increased 2-fold in cells exposed to phorbol ester for 1 h. In vitro, immunopurified STa receptor was readily phosphorylated by purified rat brain PKC. Phosphorylation was inhibited 40% by 5 microM of a synthetic peptide corresponding to the sequence around Ser1029 of the STa receptor, a site previously proposed as a potential PKC phosphorylation site. Treatment of the immunopurified STaR/GC with purified PKC increased STa-stimulated guanylyl cyclase activity 2-fold. We conclude that PKC phosphorylates and activates the STa receptor/guanylyl cyclase in vitro and in vivo; Ser1029 of the STaR/GC remains a candidate phosphorylation site by PKC.

8/7/9 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08880276 97107461
A conformational epitope in the N-terminus of the Escherichia coli heat-stable enterotoxins is involved in receptor-ligand interactions.
Garrett BM; Visweswariah SS
Centre for Reproductive Biology and Molecular Endocrinology, Indian Institute of Science, Bangalore, India.
Biochim Biophys Acta (NETHERLANDS) Nov 15 1996, 1317 (2) p149-54, ISSN 0006-3002 Journal Code: A0W
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The heat-stable enterotoxins are a family of low molecular weight, cysteine rich peptide toxins which are one of the major causes of watery diarrhea in children and adults. These toxins bind to a cell surface

receptor in intestinal cells and mediate their action through elevation of intracellular cyclic GMP. We have generated a monoclonal antibody to these peptide toxins which is able to neutralise the activity of the peptides in a human colonic cell line, the T84 cell line. The monoclonal antibody, ST:G8, appears to be directed to an epitope distinct from antibodies previously generated, and prior incubation of this antibody, or Fab generated from this antibody, with full length STh and STp peptides prevents cGMP accumulation in T84 cells. This inhibition is a direct result of the antibody preventing binding of the peptides to the receptor. ST:G8 Mab does not recognize a 13-mer biologically active analog of STp, comprising the core sequence of STp peptide, suggesting that it is directed to a region in the N-terminus of the peptides, which may modulate receptor interaction/activation. The antibody recognizes a conformational epitope in the ST peptides, since reduction and carboxyamidation of ST abolishes antibody cross-reactivity. Differential cross-reactivity of the Mab with STh and STp peptides which differ markedly only in their N-termini, suggests that this antibody recognizes a distinct conformation in the two peptides, which is essential for receptor interaction.

8/7/10 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08239766 95251707
Immunohistochemical localization of guanylin in the rat small intestine and colon.
Cohen MB; Witte DP; Hawkins JA; Currie MG
Division of Pediatric Gastroenterology, Children's Hospital Medical Center, Cincinnati, OH 45229, USA.
Biochem Biophys Res Commun (UNITED STATES) Apr 26 1995, 209 (3) p803-8 , ISSN 0006-291X Journal Code: 9Y8
Contract/Grant No.: DK 01908, DK, NIDDK
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Guanylin is an endogenous mammalian ligand which binds to guanylate cyclase C (GC-C), the Escherichia coli heat-stable enterotoxin receptor. This interaction results in intestinal Cl- and fluid secretion, which is largely, if not exclusively, mediated through the cystic fibrosis transmembrane regulator (CFTR). Using in situ hybridization, we have previously localized guanylin mRNA to villus epithelial cells of the rat small intestine and to superficial epithelial cells of the rat colon. In the present study, we demonstrate immunoreactive guanylin in a subpopulation of goblet cells in the rat jejunum and ileum. In the colon, there was immunostaining of superficial epithelial cells and goblet cells. The immunohistochemical localization of guanylin parallels the observed distribution of guanylin mRNA. Localization of guanylin in goblet cells leads us to speculate that an in vivo function of guanylin regulated, CFTR-mediated Cl- secretion is to hydrate intestinal mucin.

8/7/11 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07789939 93131979
Guanylyl cyclase C is an N-linked glycoprotein receptor that accounts for multiple heat-stable enterotoxin-binding proteins in the intestine.

Vaandrager AB; Schulz S; De Jonge HR; Garbers DL
Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas 75235.
J Biol Chem (UNITED STATES) Jan 25 1993, 268 (3) p2174-9, ISSN 0021-9258 Journal Code: HIV
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Guanylyl cyclase C (GC-C) is a newly discovered receptor found in the intestine, and possibly in other epithelia, that binds bacterial heat-stable enterotoxins (STa). The receptor has now been stably expressed in human embryonic 293 cells, which do not normally contain the receptor. Cyclic GMP concentrations are elevated 40-fold in response to 1 microM STa, and membranes obtained from the overproducing cells contain GC-C activity that can be stimulated about 9-fold by STa alone and an additional 1.4- to 2-fold by a combination of ATP and STa. The ATP effect does not appear to be due to enzyme activation, but instead to protection of GC-C against inactivation. Antibody raised against the carboxyl-terminal sequence of GC-C identified two major proteins (M(r) 140,000 and 160,000) in 293 cells expressing GC-C. Both proteins bound to wheat germ lectin-Sepharose, and N-glycosidase F treatment converted both forms to a single M(r) 120,000 protein, the size predicted from amino acid composition. The addition of high concentrations of tunicamycin to 293-GC-C cells also reduced the M(r) to 120,000, indicating that GC-C is an N-linked glycoprotein. When rat intestinal membranes or 293-GC-C cells were cross-linked with 125I-labeled STa, the major 125I-labeled protein complexes had M(r) ranging between 45,000 and 80,000. On immunoblots of rat intestinal membranes treated with a reducing agent, 3 major proteins of M(r) 65,000, 85,000, and 140,000 were specifically recognized by a GC-C antibody. However, under nonreducing conditions one major GC-C related protein appeared at a higher position (M(r) 230,000). Its mobility was reduced in the presence of STa, similar to rCG-C expressed in 293 cells. These data indicate that at least part of the lower M(r) STa-binding proteins found in intestinal extracts represent proteolytic products of GC-C.

8/7/12 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07426704 93099556
Receptors for toxic shock syndrome toxin-1 and staphylococcal enterotoxin A on human blood monocytes.
See RH; Krystal G; Chow AW
Department of Medicine, University of British Columbia, Vancouver, Canada.
Can J Microbiol (CANADA) Sep 1992, 38 (9) p937-44, ISSN 0008-4166 Journal Code: CJ3
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Staphylococcal toxic shock syndrome toxin-1 (TSST-1) as well as staphylococcal enterotoxin A (SEA) and B (SEB) have recently been shown to bind directly to the class II major histocompatibility antigen, HLA-DR. Whereas others have characterized TSST-1 and SEA binding to HLA-DR on transfected L cells or B lymphoma cell lines, we sought evidence for direct binding of TSST-1 and SEA to HLA-DR on purified human monocytes. A single class of high-affinity receptors was found for both TSST-1 (dissociation constant (Kd) 40 nM, 3.4 x 10(4) receptors per cell) and SEA (Kd 12 nM, 3.2 x 10(4) receptors per cell) on normal human monocytes. Affinity

cross-linking of 125I-labeled toxins to monocytes revealed the presence of two membrane protein subunits with molecular masses consistent with the alpha and beta chains of human HLA-DR (35 and 28 kDa, respectively). The anti-HLA-DR monoclonal antibody L243, but not L203 or 2.06, inhibited radiolabeled toxin binding to human monocytes and neutralized the mitogenic response of human T lymphocytes to both toxins. However, L243 was consistently more effective in blocking radiolabeled TSST-1 than SEA binding to human monocytes from the same donors, suggesting that TSST-1 and SEA may be binding to overlapping epitopes rather than to the same epitope on HLA-DR. Because TSST-1 and SEB bind to distinct epitopes on HLA-DR and because SEA cross competes with both TSST-1 and SEB on the HLA-DR receptor, we postulate that SEA occupies a binding site within HLA-DR that overlaps both TSST-1 and SEB.(ABSTRACT TRUNCATED AT 250 WORDS)

8/7/13 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07364948 92350150
Glycosphingolipids: the putative receptor for Staphylococcus aureus enterotoxin-B in human kidney proximal tubular cells.
Chatterjee S; Jett M
Department of Pediatrics, Johns Hopkins University, School of Medicine, Baltimore, Maryland 21205.
Mol Cell Biochem (NETHERLANDS) Jul 6 1992, 113 (1) p25-31, ISSN 0300-8177 Journal Code: NGU
Contract/Grant No.: RO-1-DK-31722, DK, NIDDK
Languages: ENGLISH
Document type: JOURNAL ARTICLE
We have investigated the binding of 125I-staphylococcal enterotoxin-B (SEB) in cultured human proximal tubular cells. We found that the binding of 125I-SEB to PT cells was time and concentration dependent and competitively inhibited by antibody against SEB. Preincubation of cells with trypsin and neuraminidase or with fetuin did not significantly impair the binding of 125I-SEB to such cells. In contrast, treatment with endoglycoceramidase completely inhibited the binding of 125I-SEB to cells. Neutral glycosphingolipids exerted a concentration-dependent inhibition of 125I-SEB binding to such cells, maximum inhibition (96% compared to control) occurred upon incubation of PT cells with neutral glycosphingolipids. Taken together, our studies indicate that SEB specifically binds to a neutral glycosphingolipid in PT cells. In contrast, staphylococcal enterotoxin-A and toxic shock toxin (TST-1) are bound to a protein in such cells.

8/7/14 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07362086 92307876
Expression of receptors for enterotoxigenic Escherichia coli during enterocytic differentiation of human polarized intestinal epithelial cells in culture.
Kerneis S; Chauviere G; Darfeuille-Michaud A; Aubel D; Coconnier MH; Joly B; Servin AL
Departement de Microbiologie et Immunologie, UFR Sciences Pharmaceutiques Paris XI, France.

Infect Immun (UNITED STATES) Jul 1992, 60 (7) p2572-80, ISSN 0019-9567 Journal Code: GO7

Languages: ENGLISH

Document type: JOURNAL ARTICLE

To study the expression of human intestinal receptors for enterotoxigenic Escherichia coli (ETEC), the human polarized intestinal epithelial cell line Caco-2 in culture and several subpopulations of HT-29 cells in culture--parental (mainly undifferentiated) HT-29 cells (HT-29 Std), an enterocytelike subpopulation obtained by selection through glucose deprivation (HT-29 Glc-), and an enterocytelike subpopulation obtained by selection through glucose deprivation which maintains its differentiation characteristics when switched back to standard glucose-containing medium (HT-29 Glc-/+)--were used. Since Caco-2 spontaneously differentiated in culture under standard culture conditions (in the presence of glucose) and HT-29 cells were undifferentiated when cultured under standard conditions (HT-29 Std) and differentiated when grown in a glucose-free medium (HT-29 Glc-), we studied the expression of the receptors for colonization factor antigens (CFA) I, II, and III and the 2230 antigen of ETEC in relation to enterocytic differentiation. We provide evidence that expression of ETEC CFA receptors develops in parallel with other differentiation functions of the cultured cells. The expression of ETEC-specific brush border receptors was studied by indirect immunofluorescence using antibodies raised against purified ETEC CFA. No ETEC receptors were detected in HT-29 Std or short-term-cultured Caco-2 cells. However, among the population of HT-29 Std cells, 2 to 4% of the cells were found to bind ETEC, and these cells expressed positive carcinoembryonic antigen immunoreactivity. This indicated that among the population of undifferentiated HT-29 cells, clusters of differentiated cells were present. ETEC CFA receptors were expressed in the apical and basolateral domains of differentiated HT-29 cells, whereas in differentiated Caco-2 cells only apical expression was observed. Both in HT-29 cells (HT-29 Glc-/+) and in Caco-2 cells cultured under standard conditions, ETEC CFA receptors develop as a function of day in culture. This indicated that the expression of the ETEC CFA receptors was a growth-related event. Indeed, ETEC CFA receptors developed in step with the apical expression of differentiation-associated proteins.

8/7/15 (Item 8 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

06996214 91373842

Effects of staphylococcal toxic shock syndrome toxin 1 on aortic endothelial cells.

Lee PK; Vercellotti GM; Deringer JR; Schlievert PM

Department of Microbiology, University of Minnesota Medical School, Minneapolis 55455-0312.

J Infect Dis (UNITED STATES) Oct 1991, 164 (4) p711-9, ISSN 0022-1899 Journal Code: IH3

Contract/Grant No.: HL-36611, HL, NHLBI; HL-33793, HL, NHLBI; CA-0913, CA , NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

In staphylococcal toxic shock syndrome, hypotension and shock due to capillary leak may rapidly lead to death of the host. To investigate its pathogenesis, the cytotoxic effects of toxic shock syndrome toxin 1 (TSST-1) on porcine aortic endothelial cells (PAEC) were examined in vitro. TSST-1 killed PAEC (as measured by 51Cr release) in a dose- and

time-dependent fashion and was blocked by anti-TSST-1 antibodies. Receptor-mediated endocytosis may be critical for the cytotoxic effects of TSST-1, as killing was inhibited by cold (4 degrees C) and by addition of chloroquine and methylamine. Furthermore, calcium and oxygen appeared necessary for TSST-1 effects on PAEC. Membrane receptor binding studies indicated PAEC bind TSST-1 with high affinity (Kd = 5.7 x 10(-7) M) and had 2.2 x 10(4) receptors/cell. Last, as measured by 125I-labeled albumin flux in a transendothelial permeability model, TSST-1 enhanced the permeability of PAEC monolayers in a dose- and time-dependent manner.

8/7/16 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06275210 86032276
Production and characterization of monoclonal antibodies against Clostridium perfringens type A enterotoxin.
Wnek AP; Strouse RJ; McClane BA
Infect Immun (UNITED STATES) Nov 1985, 50 (2) p442-8, ISSN 0019-9567 Journal Code: GO7
Contract/Grant No.: AI19844-03, AI, NIAID
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Hybridomas secreting monoclonal antibodies (MABs) specific for Clostridium perfringens type A enterotoxin were produced by fusion of P3X63Ag8.653 myeloma cells with spleen cells from BALB/c mice immunized with purified enterotoxin. Wells containing hybridomas secreting immunoglobulin G (IgG) antibodies against enterotoxin were specifically identified by an indirect enzyme-linked immunosorbent assay (ELISA), and 10 ELISA-positive hybridomas were selected and cloned twice by limiting dilution. All 10 hybridomas produced MABs containing immunoglobulin G1 heavy chains and kappa (kappa) light chains. These hybridomas were then grown as ascitic tumors in mice, and MABs were purified from the ascites fluids with DEAE Affi-gel blue. The specificity of the MABs for enterotoxin was demonstrated by immunoblotting and ELISA. Competitive radioimmunoassay with 125I-MABs suggests that these MABs recognized at least four epitopes on the enterotoxin molecule. The enterotoxin-neutralizing ability of MABs from both hybridoma culture supernatants and ascites fluids was assessed by using a 3H-nucleotide-release Vero (African green monkey kidney) cell assay. Only 2 of the 10 hybridomas produced MABs which completely (greater than 90%) neutralized the biologic activity of enterotoxin. Preincubation of 125I-enterotoxin with MABs demonstrated that MAB neutralizing ability correlated with MAB-specific inhibition of specific binding of enterotoxin to intestinal brush border membranes.

8/7/17 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05893418 90010129
Staphylococcal enterotoxin B and toxic shock syndrome toxin-1 bind to distinct sites on HLA-DR and HLA-DQ molecules.
Scholl PR; Diez A; Geha RS
Division of Allergy and Immunology, Children's Hospital, Boston, MA.
J Immunol (UNITED STATES) Oct 15 1989, 143 (8) p2583-8, ISSN 0022-1767 Journal Code: IFB

Contract/Grant No.: AD07321-01, AD, ADAMHA; AI20373-05, AI, NIAID
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Staphylococcal enterotoxins (SE) activate human T cells in vitro. This requires the presence of Ia+ accessory cells but does not require processing of the toxin by the accessory cell. We and others have recently demonstrated direct binding of SE to human MHC class II molecules. In this study, we have compared in detail the ability of class II molecules to bind two SE, toxic shock syndrome toxin-1 (TSST-1) and SEB. Scatchard analysis of equilibrium binding data indicate that SEB binds to Ia+ human cell lines with a 10-fold lower affinity than TSST-1. Likewise, SEB precipitates HLA-DR alpha- and beta-chains from detergent lysates of Ia+ cells less efficiently than TSST-1. The binding of TSST-1 and SEB to transfected L cells expressing HLA-DR and HLA-DQ gene products was differentially inhibited by anti-HLA-DR mAb. There was virtually no cross-inhibition of TSST-1 and SEB in competitive binding assays. We conclude that TSST-1 and SEB bind to two MHC class II sites which can be distinguished by their relative accessibility to blocking by anti-class II mAb and heterologous toxin.

8/7/18 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05222210 86313709

Anti-idiotypic antibodies that inhibit immediate-type skin reactions in unsensitized monkeys on challenge with staphylococcal enterotoxin.

Bamberger U; Scheuber PH; Sailer-Kramer B; Bartsch K; Hartmann A; Beck G; Hammer DK

Proc Natl Acad Sci U S A (UNITED STATES) Sep 1986, 83 (18) p7054-8, ISSN 0027-8424 Journal Code: PV3
Languages: ENGLISH
Document type: JOURNAL ARTICLE

The staphylococcal enterotoxin B (SEB)-induced immediate-type skin reaction in unsensitized monkeys was used as a nonimmunological mast cell stimulus to examine whether the toxin exerts its effect via specific receptors on the target cell membrane. Anti-idiotypic antibodies (anti-Id) were raised in BALB/c mice against monoclonal anti-SEB antibodies (anti-SEB) and purified by idiotype affinity chromatography. The anti-Id nature of the antibody was demonstrated by its ability to inhibit the binding of 125I-labeled anti-SEB to the ligand in a concentration-dependent manner. Moreover, binding of anti-SEB to anti-Id was antagonized by the SEB ligand in a competitive way. These antibodies completely abolished skin reactions in unsensitized monkeys on challenge with SEB and impeded those provoked by staphylococcal enterotoxins A and C1 but did not have the biological activity of the toxin. These data are compatible with the view that receptors for staphylococcal enterotoxins may exist on the membrane of mast cells in the skin of unsensitized monkeys. The data suggest an experimental approach for producing anti-cell receptor antibodies that are of potential value to influence the course of staphylococcal enterotoxin-mediated effects.

8/7/19 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

04863916 86154509
Inhibition of small intestinal colonization of enterotoxigenic Escherichia coli by streptococcus faecium M74 in pigs.
Ushe TC; Nagy B
Zentralbl Bakteriol Mikrobiol Hyg [B] (GERMANY, WEST) Dec 1985, 181 (3-5) p374-82, ISSN 0174-3015 Journal Code: Y5R
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Colonizing and anti E. coli activity of S. faecium M74 was tested by oral inoculation of cesarean derived, colostrum deprived piglets with Streptococcus faecium M74 and subsequently with a heat stable enterotoxin (ST) producing E. coli (O101 : K30 : K99 : NM). Enterotoxin neutralization and co-culture studies were also performed in vitro. Bacterial counts in 10 cm ileal segments, fluorescein antibody stained cryostat sections, as well as 0.5 micron sections from the ilea of the experimental pigs taken 16 hours post exposure to enterotoxic E. coli (ETEC) all indicated that S. faecium M74 could not colonize the ileum of the newborn pigs, in a single high (7 X 10(8) - 3 X 10(10)) dose either in TSB or in milk suspension, in contrast to the ETEC. However, S. faecium given in milk suspension resulted a marked decrease in ileal colonization of ETEC and in weight loss of piglets. In vitro experiments indicated that neither extracellular nor cell-bound products of S. faecium M74 could neutralise ST, but there was a significant reduction of pH in the TSB cultures of S. faecium that was accompanied by a reduction in ETEC counts of the mixed cultures.

8/7/20 (Item 1 from file: 156)
DIALOG(R)File 156:Toxline(R)

02256152 Subfile: CRISP-96-AI13600-17
IMMUNOBIOLOGY OF LY+ T-CELL SUBCLASSES
CANTOR HI
DANA-FARBER CANCER INSTITUTE, 44 BINNEY STREET, D730, BOSTON, MA 02115
Source: Crisp Data Base National Institutes Of Health
Language: ENGLISH
Document Type: Research
Spon. Agency: U.S. DEPT. OF HEALTH AND HUMAN SERVICES; PUBLIC HEALTH SERVICE; NATIONAL INST. OF HEALTH, NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
Contract Number: 5R01AI13600-17
Award Type: Grant
RPROJ/CRISP The proposed research is aimed at defining fundamental mechanisms responsible for the development and function of the CD4 T-cell subset. We propose studies to define the mechanisms of intrathymic commitment to the CD4 lineage, alternative signalling pathways coupled to TCR ligation of CD4 cells by superantigen or conventional antigen and the role of the CD4 molecule in regulating the response of these cells to TCR ligation. <SA1- Our approach to defining the mechanism of CD4 commitment uses thymocytes from mice deficient in a class II-dependent instructional signal. We propose experiments to test the hypothesis that committed CD4 transitional thymocytes are generated stochastically and require a CD4-dependent signal for further development. <SA2,3- We have obtained considerable evidence that two functionally distinct signal transduction pathways are coupled to the TCR using dual- reactive (superantigen/peptide) CD4 clones. We propose to further define the biochemical elements of the two pathways and to delineate the interaction between the TCR and the two types of ligand that may be coupled to the two signalling pathways. <SA4-

We propose studies to extend our analyses of unresponsiveness in dual-reactive CD4 clones to the more physiological response of primary CD4 T-cells. One approach uses primary CD4 cells which express the 2B4 TCR transgene. These cells display the differential response phenotype after stimulation in vitro by superantigen (anergy) and peptide antigen (responsiveness) noted in earlier studies of dual-reactive CD4 clones. We will use this system to define the cellular and molecular mechanism of superantigen-induced unresponsiveness in primary CD4 cells. A second set of studies examines the mechanism of negative signalling by the CD4 molecule in primary CD4 T cells. We propose to define the contribution of the cytoplasmic (CYT) domain of the CD4 molecule to negative signalling in experiments which compare the regulatory effects of CD4- derived CYT domains with CD8-derived CYT domains. <SA5- Recognition of Staph enterotoxins (SEs) by CD4 T cells is usually associated with presentation of these ligands by class II products. We have recently identified a subgroup of SEs which represent exceptions to this rule. We propose studies to distinguish the potential antigen-presenting role of non-class II SEC/SEE receptors from the role of direct TCR ligation by these SEs.

8/7/21 (Item 1 from file: 351)
DIALOG(R)File 351:DERWENT WPI

009578637 WPI Acc No: 93-272183/34
XRAM Acc No: C93-121439
New purified enterotoxin receptor protein - used to develop prods. for treating abnormal conditions caused by bacterially released enterotoxin, partic. diarrhoea
Patent Assignee: (UYVA-) UNIV VANDERBILT
Author (Inventor): GARBERS D L; SCHULZ S
Number of Patents: 001
Number of Countries: 001
Patent Family:

CC Number	Kind	Date	Week	
US 5237051	A	930817	9334	(Basic)

Priority Data (CC No Date): US 623033 (901206)
Abstract (Basic): US 5237051 A

A new purified, isolated protein has a biological property of binding heat stable enterotoxin and has guanylyl cyclase activity, the protein contg. a single putative transmembrane domain and an intracellular portion including a protein kinase domain and a cylase catalytic domain, the protein being derived from a clone pSVL-GCC deposited as ATCC 68482.

More specifically, the protein has the amino acid sequence shown or is encoded by a DNA which is capable of hybridising to a DNA encoding the amino acid sequence shown under moderately stringent washing conditions.

USE - The protein is an enterotoxin receptor which can be used as a therapeutic to control intestinal fluid permeation as well as abnormal conditions caused by bacterially released enterotoxin. In partic., the binding domain of the protein or antibodies to the protein can be used to eliminate diarrhoea. The protein can also be used to isolate ligands and to screen for antagonists of toxin binding. Dwg. 0/5

Derwent Class: B04; D16;
Int Pat Class: C07K-013/00; C12N-015/12

8/7/22 (Item 1 from file: 434)
DIALOG(R)File 434:Scisearch(R) Cited Ref Sci

14854744 Genuine Article#: UQ952 Number of References: 23
Title: SUBUNITS OF SOLUBLE GUANYLYL CYCLASE IN RAT AND GUINEA-PIG SENSORY GANGLIA
Author(s): KUMMER W; BEHRENDS S; SCHWARZLMULLER T; FISCHER A; KOESLING D
Corporate Source: UNIV GIESSEN,INST ANAT & CELL BIOL,AULWEG 123/D-35385 GIESSEN//GERMANY/; FREE UNIV BERLIN,INST PHARMACOL & TOXICOL/W-1000 BERLIN//GERMANY/; UNIV MARBURG,INST ANAT & CELL BIOL/W-3550 MARBURG//GERMANY/
Journal: BRAIN RESEARCH, 1996, V721, N1-2 (MAY 20), P191-195
ISSN: 0006-8993
Language: ENGLISH Document Type: ARTICLE
Abstract: Soluble guanylyl cyclase is a heterodimeric (alpha,beta) enzyme generating the second messenger, cGMP, upon activation by the gaseous messenger, nitric oxide. The occurrence and distribution of alpha(1)-, alpha(2)-, beta(1)- and beta(2)-subunits were investigated in trigeminal and dorsal root ganglia on the mRNA and the protein level. Reverse transcription PCR analysis demonstrated mRNA coding for alpha(1)-, alpha(2)-, and beta(1)-subunits in guinea-pig trigeminal and dorsal root ganglia. In agreement with these data, immunoreactivity to the alpha(1)-subunit was found in satellite and Schwann cells, while alpha(2)-subunit immunoreactivity was localized to axons of large diameter. The distribution of the beta(1)-subunit could not be studied on the protein level since the antiserum was ineffective in immunohistochemistry. However, previous studies and the RT-PCR data argue in favour of alpha(1)/beta(1)- and alpha(2)/beta(1)-heterodimerization and colocalization. In both species, beta(2)-subunit immunoreactivity was confined to neuronal perikarya, primarily of large diameter. Although these results were obtained with two different antibodies directed against different epitopes, the corresponding mRNA could not be detected by RT-PCR analysis. The reason for this discrepancy remains unclear, at present, but could be explained by a variant beta(2)- or highly homologous as yet unidentified beta-subunit. This study demonstrates the presence of soluble guanylyl cyclase in sensory ganglia with a differential, cell type-specific distribution of the individual subunits.

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Title: HUMAN MAJOR HISTOCOMPATIBILITY COMPLEX CLASS-II-NEGATIVE COLON-CARCINOMA CELLS PRESENT STAPHYLOCOCCAL SUPERANTIGENS TO CYTOTOXIC LYMPHOCYTES-T - EVIDENCE FOR A NOVEL ENTEROTOXIN RECEPTOR
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Abstract: The staphylococcal enterotoxins (SE) bind to major histocompatibility complex (MHC) class II molecules on target cells and activate T cells expressing particular T cell receptor V-beta-sequences. In this report we demonstrate that SE bind to the MHC class II- SW620, Colo320DM and WiDr human colon carcinoma cell lines and direct cytotoxic T lymphocytes (CTL) to mediate strong target cell killing. Flow cytometry analysis, immunoprecipitation and Northern blotting experiments failed to demonstrate any surface expression of HLA-DR, HLA-DP and HLA-DQ isotypes on the SW620 colon carcinoma cell line, whereas abundant expression of these isotypes was seen on Raji cells. SEB and SEC1 were efficiently presented at picomolar concentration by the MHC class II- colon carcinoma cells and MHC class II+ Raji cells, whereas SEA and SED were preferentially presented on the MHC class II+ Raji cells. An anti-HLA-DR monoclonal antibody inhibited SEB-induced CTL targeting to Raji, but did not influence the killing of SW620 cells. Our data suggests the existence of functionally active SE-binding structures on human colon carcinoma cells which are distinct from the conventional MHC class II molecules. The possibility that these putative new SE receptors play a role in the enterotoxin action of SE must be considered.
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